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Determinants of plasma copeptin: a systematic investigation in a pediatric mechanical ventilation model

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Abstract (149 words)

Copeptin, the C-terminal part of the arginine vasopressin precursor peptide, holds promise as a diagnostic and prognostic plasma biomarker in various acute clinical conditions. Factors influencing copeptin response in the critical care setting are only partially established and have not been investigated systematically. Using an *in vivo* infant ventilation model (Wistar rats, 14 days old), we studied the influence of commonly occurring stressors in critically ill children. In unstressed ventilated rats basal median copeptin concentration was 22 pmol/L. In response to respiratory alkalosis copeptin increased 5-fold, while exposure to hypoxemia, high PEEP, hemorrhage, and psycho-emotional stress produced a more than 10-fold increase. Additionally, we did not find a direct association between copeptin and acidosis, hypercapnia, and hyperthermia. Clinicians working in the acute critical care setting should be aware of factors influencing copeptin plasma concentrations. Moreover, our results do have implications for animal studies in the field of stress research.

Keywords

Arginine vasopressin; copeptin; critical care; mechanical ventilation; positive end-expiratory pressure; stress response

1. Introduction

Arginine vasopressin (AVP), also known as antidiuretic hormone (ADH), is a neurohypophysial hormone playing a key role in osmoregulation and water homeostasis. Moreover, non-osmotic release of AVP is described in adult patients with cardiovascular and renal diseases (Stoiser et al., 2006; Meijer et al., 2011), stroke (Katan et al., 2009), sepsis (Morgenthaler et al., 2007; Jochberger et al., 2009), and pneumonia (Müller et al., 2007). In contrast to AVP, the C-terminal part of provasopressin, copeptin, is both without known physiological activity and easy and robust to measure (Morgenthaler et al., 2006). Since copeptin is released equimolar to AVP, there is considerable interest in copeptin as a diagnostic and prognostic biomarker (Katan and Christ-Crain, 2010; Mellbin et al., 2010; Potocki et al., 2010). Ideally, biomarkers are surrogate markers for disease and its severity (Vasan, 2006).

Recently, copeptin has also been shown to be a highly sensitive though minimally specific marker of fetal and neonatal stress (Wellmann et al., 2010; Schlapbach et al., 2011; Benzing et al., 2011). As opposed to adults with inappropriately low AVP in septic shock, studies in children with septic shock did not find AVP deficiency (Leclerc et al., 2003; Lodha et al., 2006). Various reasons may account for this inconsistence; firstly, well known age-related differences in predisposing diseases, physiology, and management strategies; secondly, methodological issues since AVP measurement is cumbersome when compared to copeptin; finally, heterogeneity of critical care patients usually affected by more than one pathophysiological disorder making it difficult to allocate AVP response.

Before implementing copeptin in the field of pediatric critical care, factors affecting its plasma concentration should be systematically investigated. Hence, the aim of this project was to study copeptin plasma concentrations in response to commonly

occurring stressors and to call for caution when elevated copeptin levels are measured and interpreted in pediatric critical care practice. Apart from the advantage of protocol standardization, using an *in vivo* experimental infant (non-neonatal) rat model allowed investigation of selected pathophysiological conditions that can generally not be examined and partitioned in the critical care context. We hypothesized that copeptin plasma concentration is affected by mechanical ventilation, hypovolemia, hypoxemia, acidosis, and hyperthermia.

2. Methods

2.1 Animals

Experimental protocols were approved by the cantonal authority of Zurich and were conducted in compliance with legal requirements and the Swiss Animal Welfare Legislation. Infant Wistar rats of either sex were purchased from Charles River Laboratories International, Inc. (Sulzfeld, Germany) at the age of ~7 days and were used for experimentation at the age of 14 days. Dams were provided food and water ad libitum and were kept under 12 h light dark cycles.

2.2 Animal preparation before mechanical ventilation

After brief inhalational anesthesia with isoflurane, rats were weighed and anesthetized with an intraperitoneal (i.p.) injection of a solution containing ketamine (75 μg/g body weight, BW) and xylazine (12 μg/g BW). Then a tracheostomy was performed and a 10 mm polyethylene cannula inserted (ID: 0.86 mm). Rats were then placed in supine position on a heating mat and connected to a computer-controlled ventilator (*flexiVent*®, Scireq, Montreal, Canada) using the following settings: tidal volume (V_T) ~8.0 mL/kg, positive end-expiratory pressure (PEEP) 5 cmH_2O, respiratory rate (RR) 90/min, and inspired oxygen fraction (FiO_2) 0.4. Lung volume history was standardized by two lung volume recruitment maneuvers (up to 40 cmH_2O with 9-s ramp and 3-s plateau) within 2 min. Peak and mean airway opening pressures (P_{ao}) were displayed by the *flexiVent*® system. Heart rate (HR) and oxygen saturation (SpO_2) were monitored via an animal pulse oximeter (*MouseOx*^{TM}, STARR Life Sciences Corporation^{TM}, Oakmont, PA, USA) by placing a non-invasive sensor on the tail. Peak and mean P_{ao}, HR, SpO_2 and body temperature were monitored and again

recorded at the end of the protocol. Non-ventilated controls underwent the same anaesthetic and surgical procedures but were not connected to the ventilator.

2.3 Allocation to study groups and experimental protocol

Except for non-ventilated controls (n = 10 animals per group) all animals were ventilated for 120 min (Fig. 1). Infant rats were randomly allocated to the following study groups: "ventilated controls" (n = 9), i.e. mechanical ventilation without additional stressors applying V_T ~8 mL/kg, PEEP 5, FiO_2 0.4, RR 90/min, and constant body temperature at 38.0˚C; "acidosis" and "alkalosis" (both n = 10), i.e. respiratory acidosis and alkalosis induced via RR of 60/min and 180/min, respectively; "hypoxemia" (n = 10) and "hyperoxemia" (n = 9) induced via FiO_2 of 0.21 and 1.0, respectively; "PEEP 1" (n = 10) and "PEEP 9" (n = 9), respectively, where PEEP levels were regulated by the depth of the expiratory line from the ventilator into a water column, also, RR in the PEEP 9 group was set at 120/min in order to achieve normal blood gas values; "hypothermia" and "hyperthermia" (both n = 10) induced via body temperature of 33˚C and 41˚C, respectively; "AH" and "AHFR", i.e. acute hemorrhage without (n = 8) and with subsequent fluid resuscitation (n = 7), respectively. Acute hemorrhage was produced after 90 min of protective mechanical ventilation via transcutaneous cardiac puncture and withdrawal of ~0.38 mL blood within 30 s. Blood volume was supposed to approximate 7% of body weight (Cannizzaro et al., 2010). Therefore, removing ~0.38 mL blood resulted in a controlled hemorrhage of ~15% estimated blood volume. Both vital and respiratory parameters were carefully monitored during and after this intervention. Animals in group "AHFR" subsequently received fluid resuscitation via i.p. application of ~0.75 mL saline.

Body temperature was controlled and kept constant by a thermocouple feedback rectal sensor connected to a heating mat (Physitemp Instruments, Incorporation, TCAT-2LV Temperature Controller, Clifton, New Jersey, USA). Before starting to collect and record urine output, following connection to the ventilator the bladder was emptied by delicately pressing on the fundus. After that, urine was either obtained via gently pressing on the bladder if supposed to be full or via direct bladder puncture at the end of the protocol.

To avoid dehydration, all ventilated animals received i.p. 0.5 mL 0.9% saline at the time point 30 min. An anesthetic top-up with ketamine/xylazine was given ~15 min before the end of the protocol. Discomfort, stress, or pain was assessed by changes in heart rate, breathing efforts, and reaction to paw pinch reflex.

2.4 Sampling and processing of blood for copeptin and blood gas analysis

Before disconnecting infant rats from the ventilator blood was withdrawn directly from the heart following partial laparotomy and sternotomy. Few drops were used for blood gas analysis (i-STAT®, Axonlab, Abbott Laboratories, Illinois, USA). Blood gas analyses were performed in ventilated animals only. Also, cartridges for lactate measurements were not available for all infant rats. Hence, we focused on those groups where we expected significant differences when compared to ventilated controls, i.e. hypoxemia n = 10; hyperthermia n = 9; hypothermia n = 7; ventilated controls, respiratory alkalosis, and "AH", n = 6 respectively; hyperoxemia and "AHFR", n = 5, respectively; respiratory acidosis n = 4; PEEP 9 n = 3; PEEP 1 n = 2.

The remaining blood volume was stored at 4˚C until centrifugation at 3'000 x g for 10 min at room temperature. Plasma was then transferred in a new EDTA tube and stored at –80˚C until copeptin analysis. In animals from groups "AH" and "AHFR" copeptin

was measured twice, once from blood withdrawal at the time point 90 min and once 30 min later, i.e. at the end of the protocol.

2.5 Copeptin analysis

Measurement of plasma copeptin was done in a single batch with a research sandwich immunoluminometric assay (B.R.A.H.M.S C-terminal pro-AVP luminescence immunoassay; B.R.A.H.M.S GmbH, Hennigsdorf, Germany). As the assay developed for the detection of human plasma copeptin (Morgenthaler et al., 2006) was not sufficiently cross-reactive with rat plasma copeptin, the tracer antibody of the assay was replaced by a purified sheep antibody developed against a peptide corresponding to amino acid positions 156-168 of rat pre-pro-vasopressin, and dilutions of a peptide corresponding to amino acid positions 136-168 of rat pre-pro-vasopressin were used as calibrators. All other assay conditions were the same as described for the human copeptin assay.

2.6 Statistical analysis

One-way ANOVA with Holm-Sidak post hoc tests were used for group comparisons of copeptin, SpO_2, HR, peak and mean P_{ao}, pH, pCO_2, lactate, urine output, and weight. For comparison of amount of blood withdrawn to induce acute hemorrhage in groups “AH” and “AHFR” t-test was used. Two-way repeated measurement ANOVA with Holm-Sidak post hoc analysis was used to compare copeptin concentrations before and after acute hemorrhage in animals from groups “AH” and “AHFR”. Data were transformed where appropriate to ensure the assumptions of normality and equal variance were satisfied. Where this was not possible equivalent non-parametric comparisons were used. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Baseline values (group means)

Between all groups no statistically significant differences were found in relation to body weight (34.5-38.7 g; p = 0.59), SpO_2 (98.3-99.0%; p = 0.06), heart rate (281-298/min; p = 0.84), peak P_{ao} (11.0-12.1 cmH_2O; p = 0.75), and mean P_{ao} (7.9-8.4 cmH_2O; p = 0.59).

3.2 Vital signs, ventilation parameters, blood gas, lactate, and urine output at the end of the study

Values of SpO_2, heart rate, peak and mean P_{ao}, pH, pCO_2, lactate, and urine output are shown in Table 1. Mean body temperatures in groups "hypothermia" and "hyperthermia" reached 32.8˚C (SD 1.6˚C) and 40.7˚C (SD 0.6˚C), respectively.

3.3 Copeptin concentrations at the end of the study

Figure 2 illustrates copeptin plasma concentrations in all mechanically ventilated infant rats. Except for the acute hemorrhage groups "AH" and "AHFR" (p = 0.49), we found statistically significant differences between all studied pairs. At the end of the protocol copeptin values of infant rats subjected to respiratory alkalosis, hypoxemia, PEEP 9, and acute hemorrhage were statistically significantly increased when compared to ventilated controls.

3.4 Copeptin response following acute hemorrhage

Figure 3A shows copeptin values before and 30 min after induction of acute hemorrhage. The amount of blood withdrawn was similar between groups, i.e. 0.38

mL (SD 0.04 mL) and 0.39 mL (SD 0.05 mL) in groups “AH” and “AHFR”, respectively ($p = 0.85$). The copeptin rise was statistically significant and independent of subsequent fluid resuscitation.

3.5 Copeptin half life

Figure 3B demonstrates the course of copeptin based on plasma concentrations obtained at the time points 0, 90, and 120 min. Copeptin levels in non-ventilated rats were ~10-fold higher when compared to ventilated controls (median value of 227 pmol/L versus 22 pmol/L). Non-ventilated controls underwent anesthesia, tracheostomy, and intubation without mechanical ventilation at the time point 0 min. Groups “AH” and “AHFR” were protectively ventilated up to the time point 90 min where blood was withdrawn to induce acute hemorrhage. Lastly, infant rats from the ventilated control group were mechanically ventilated throughout the protocol without any additional stressor. Copeptin concentrations significantly decreased over time. Based on the mean concentrations of our 3 time points and the following formula: T ½ = (log 2 x elapsed time) / log (concentration at the beginning / concentration at the end), we calculated a half life ranging from 23-47 min.

3.6 Copeptin and gender

Gender comparison revealed no statistically significant difference between all studied groups (p in all cases > 0.11), except for acute hemorrhage without fluid resuscitation (AH) where females showed higher copeptin levels ($p = 0.044$). However, it needs to be mentioned that this difference is based on comparison of 3 males and 5 females only.

4. Discussion

Our results demonstrate that knowledge of potential stressors is mandatory in order to interpret the sensitive and early responsive biomarker copeptin. Using an *in vivo* infant rat model we were able to both standardise protocols and systematically examine some of the commonly occurring stressors in intensive care units. Also, to the best of our knowledge, this is the first study detecting plasma rat copeptin concentrations quantitatively. Our major findings are summarised as follows: first, non-osmotic stimuli such as respiratory alkalosis, hypoxemia, and acute hemorrhage produced significant copeptin responses, whereas respiratory acidosis, hyperoxia, and changes in body temperature did not; second, non-injurious mechanical ventilation per se did not stimulate copeptin release; and third, supposedly unstressed non-ventilated controls showed clearly increased copeptin values.

Acidosis has been considered a stimulus for AVP release (Holmes et al., 2001). In our study, the role of pH was investigated via induction of hyper- and hypocapnia. For two main reasons we decided to alter pH by ventilation only. First, respiratory acidosis and alkalosis can be achieved easily and rapidly in artificially ventilated animals. Second, relevant metabolic compensation is unlikely to occur within 2 h. Acidosis as a stimulus for AVP release originates from the work by Wood and Chen (1989) who used an i.v. infusion of hydrochloric acid to induce metabolic acidosis in fetal sheep. Later studies (Rose et al., 1984; Raff et al., 1991) presented a correlation between hypercapnic acidosis and AVP, however only in combination with hypoxemia. Based on our study results we question whether there is enough evidence to support that both acidosis and hypercapnia (without hypoxemia) play a key role in AVP release. In contrast to our hypothesis, we found that hypocapnic alkalosis caused

a significant increase of copeptin. As to the association between copeptin and hypocapnic alkalosis we can only speculate since the finding was unexpected and to our knowledge not studied so far. The mild but significant increase in lactate in the context of (normoxic) hypocapnic alkalosis is well-known (Eldridge and Salzer, 1967) and unlikely the cause of the observed copeptin increase. From a physiological point of view, hypocapnia induces cerebral vasoconstriction with a subsequent decrease in cerebral blood flow. Whether hypocapnia of ~3.5 kPa, as in our model, was sufficient to produce significant cerebral ischemia remains unclear, but could provide a possible explanation for copeptin elevation (Katan et al., 2009).

As hypothesised, hypoxemia resulted in a significant copeptin increase. This finding was in line with previous studies reporting major AVP release following normo- and hypercapnic hypoxemia (Anderson et al., 1978; Smith et al., 1995). In fact, hypoxemia can be considered a powerful stressor compromising homeostatic balance. Afferents from chemo- and baroreceptors are supposed to mediate AVP release via activation of both hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system (Anderson et al., 1978; Schrier et al., 1979; Leng et al., 1999; Goldstein and Kopin, 2008).

Based on the fact that positive pressure ventilation alters hemodynamics and renal function (Pannu and Mehta, 2007) we hypothesised that any ventilation strategy would increment AVP release. In order to investigate the impact of mechanical ventilation on AVP response we applied a protective ventilation strategy. In addition, we used two strategies with lower and higher positive end-expiratory pressure (PEEP) to mimic particularly low and high intrathoracic pressures (ITP), respectively. As

opposed to our hypothesis, artificial ventilation per se did not stimulate AVP. However, application of high PEEP resulting in elevated peak and mean airway opening pressures, and hence increased ITP, produced a significant copeptin rise. This finding is important and clinically relevant since ventilation with high PEEP levels is frequently applied as part of the "open lung" strategy. From a physiological point of view our result can be explained as follows. Mechanical ventilation with high PEEP increases both ITP and lung volume. ITP reduces venous return to the right atrium. At the same time, increased airway pressure raises extraluminal pressure of alveolar capillaries resulting in elevated right ventricular afterload. Consequently, right and left sided stroke volumes decrease (Feihl and Broccard, 2009). This hemodynamic change is then detected by low (atria and ventricles) and high (aorta and carotis) pressure mechanoreceptors. Decrease in preload and blood pressure "unloads" these receptors hereby increasing AVP secretion via afferent signals to the HPA axis (Koyner and Murray, 2010). Furthermore, significantly reduced urine output can be attributed to a neurohumoral response where increased sympathetic tone and activation of the renin-angiotensin-axis lead to decreased renal blood flow and eventually oliguria.

Similarly to hypoxemia, severe hyper- and hypothermia are intense threats to homeostasis. Also, a potential role of AVP in rat thermoregulation has been suggested by Jasnic et al. (2010). In the critical care context hyperthermia is often encountered in association with inflammation, infection, or drugs, while controlled hypothermia has become a therapeutic approach, e.g. following cardiac arrest in adults or hypoxic-ischemic encephalopathy in newborns. In our model we were able to produce significant tachycardia and bradycardia in hyperthermia and hypothermia groups,

respectively (Table 1). Though we found small but statistically significant differences in copeptin plasma concentrations between hyper- and hypothermia, no copeptin differences were found when compared to the ventilated normothermic controls (Fig. 2). It is conceivable that externally induced hyperthermia cannot be compared to infection-induced fever in terms of homeostatic threat. Additionally, it is possible that our hyperthermia model was inadequate to induce physiologically relevant alterations since significant dehydration with decreased urine output (Table 1) and subsequent AVP/copeptin release did not occur. On the other hand, unaltered copeptin concentrations in response to severe hypothermia corroborate clinical findings obtained in neurosurgical adult patients showing no effect of hypothermia on AVP level (Chi et al. 2001). These findings suggest that in both anesthetised rats and humans, moderate to severe hypothermia is not a relevant stressor for AVP/copeptin release.

AVP is essential in maintaining blood pressure. Changes in blood volume or pressure have previously been shown to strongly activate AVP release (Bisset and Chowdrey, 1988; Leng et al., 1999; Morgenthaler et al., 2007). As expected, in our model moderate hemorrhage accompanied by a significant rise of lactate caused a rapid jump in copeptin concentration. Similarly to the high PEEP-related change in hemodynamics, presumably relaxation of low and high pressure mechanoreceptors in the heart chambers and aortic arch / carotid body, respectively, played a major role. These cardiovascular receptors most likely led to an alteration of discharge rate resulting in a stress response via afferent fibres to the HPA axis (Bisset and Chowdrey, 1988). In contrast to our hypothesis, immediate fluid resuscitation did not mitigate early copeptin response. This finding probably reflects the fact that a

powerful and life threatening stimulus activating the stress response-cascade cannot be reversed or weakened in an early phase.

Copeptin concentrations measured in non-ventilated control groups were completely unexpected. What distinguishes non-ventilated infant rats from all other groups is the time point of blood withdrawal. In other words, though we did not measure copeptin in ventilated infant rats at the time point 0 min, it is conceivable that due the strict study protocol, i.e. animal handling and preparation, these animals experienced similar emotional stress before group allocation and ventilation. Moreover, protective mechanical ventilation under anesthesia and without additional stressors for 120 min offered an opportunity to investigate copeptin half life.

On average, pups were separated from their dam and littermates for ~30 min before starting inhalational anesthesia followed by i.p. anesthesia and subsequent blood withdrawal. A surgical procedure can be excluded as cause of elevated copeptin at the time point 0 min since blood withdrawal occurred after i.p. anesthesia. Moreover, we cannot exclude that pharmacological agents may have influenced copeptin concentration. On the other hand, anesthetic top-ups applied ~15 min before the end of the protocol did not result in copeptin increase. Furthermore, animal handling was done by experienced persons in compliance with accepted international guidelines. Though there is enough evidence showing that "stress" has the potential to activate the HPA axis and sympathetic nervous system (Charmandari et al., 2005; Goldstein and Kopin, 2008), it was not evident to us that our infants rats were exposed to emotional stress. However, when taking into account that both maternal separation and exposure to an unknown environment is undoubtedly stressful we might have to reconsider the concept of expected versus perceived emotional stress.

Given that infant rats experienced a rather short period of “stress”, one is tempted to speculate that the perceived stress must have been major. If this assumption holds true there might be implications for animal handling and studies in the field of emotional stress response perhaps requiring longer adaptation training and time.

Recently, it has been argued that gender has to be taken into consideration when interpreting copeptin levels. Bhandari et al. (2009) found higher copeptin concentrations in healthy male volunteers when compared with females. In our study, we did not find significant correlations between gender and copeptin. Given the major differences in study design and age (adult human vs infant rat), and severity of disease (healthy volunteers vs “sick” infants), it is virtually impossible to draw conclusions for clinical practice.

The following study limitations have to be acknowledged. Like all models of disease, results from a rat model can only cautiously be extrapolated to humans. On the other hand, strict standardisation and investigation of “single” factors can only be achieved by an *in vivo* animal model. In terms of rat-adjusted copeptin assay, we have to acknowledge that as opposed to human studies we did not measure AVP in parallel with copeptin. To correct this shortcoming, future rat studies should correlate copeptin with AVP. Due to our experimental set-up it remains unclear whether the copeptin elevation in hypocapnic alkalosis is attributable to pH or hypocapnia. Investigating the impact of metabolic alkalosis on copeptin was beyond the scope of this study, but might be an interesting starting point for future studies. In terms of model selection it might have been helpful to induce fever in addition to our hyperthermia model where heat was applied externally. Moreover, measurement of

plasma osmolarity and electrolytes might have provided useful information regarding status of dehydration. As to the hemorrhage model we did not measure blood pressure to objectify response to both blood withdrawal and fluid resuscitation. However, we are confident that hemodynamic changes were significant since we observed a hyperacute and temporary loss of SpO_2 in all animals. Moreover, in order to differentiate between endocrine and emotional stress (Katan and Christ-Crain, 2010), we propose that future studies additionally measure cortisol concentration in plasma or urine. Lastly, due to the protocol duration we were not able to perform serial copeptin measurements allowing documentation of its dynamics and longer-term course.

In conclusion, we have shown that copeptin is significantly influenced by respiratory alkalosis, hypoxemia, high PEEP, hemorrhage, and psycho-emotional stress. Moreover, in contrast to previous studies we cannot confirm a direct association between copeptin and acidosis, hypercapnia, and hyperthermia. Physicians working in the acute critical care setting should be aware of these factors when interpreting copeptin plasma concentrations. Finally, our results do have implications for animal studies in the field of stress research.

5. Disclosure

J. Struck is employed by BRAHMS GmbH, Hennigsdorf, Germany, the company manufacturing the copeptin assay for which it owns patent rights. The present study was not designed and financed by Thermo Fisher Scientific, BRAHMS GmbH. The remaining authors report no conflict of interest.

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7. References

Anderson, R.J., Pluss, R.G., Berns, A.S., Jackson, J.T., Arnold, P.E., Schrier, R.W., McDonald, K.E., 1978. Mechanism of effect of hypoxia on renal water excretion. J. Clin. Invest. 62, 769-777.

Bhandari, S.S., Loke, I., Davies, J.E., Squire, I.B., Struck, J., NG, L.L., 2009. Gender and renal function influence plasma levels of copeptin in healthy individuals. Clin. Sci. (Lond.). 116, 257-263.

Benzing, J., Wellmann, S., Achini, F., Letzner, J., Burkhardt, T., Beinder, E., Morgenthaler, N.G., Haagen, U., Bucher, H.U., Bührer, C., Lapaire, O., Szinnai, G., 2011. Plasma copeptin in preterm infants: a highly sensitive marker of fetal and neonatal stress. J. Clin. Endocrinol. Metab. 96, E982-E985.

Bisset, G.W., Chowdrey, H.S., 1988. Control of release of vasopressin by neuroendocrine reflexes. Q. J. Exp. Physiol. 73, 811-872.

Cannizzaro, V., Berry, L.J., Nicholls, P.K., Hantos, Z., Sly, P.D., 2010. High tidal volume ventilation is not deleterious in infant rats exposed to severe hemorrhage. J. Trauma. 69, E24-E31.

Charmandari, E., Tsigos, C., Chrousos, G., 2005. Endocrinology of the stress response. Annu. Rev. Physiol. 67, 259-284.

Chi, O.Z., Choi, Y.K., Lee, D.I., Kim, Y.S., Lee, I., 2001. Intraoperative mild hypothermia does not increase the plasma concentration of stress hormones during neurosurgery. Can. J. Anaesth. 48, 815-818.

Eldridge, F., Salzer, J., 1967. Effect of respiratory alkalosis on blood lactate and pyruvate in humans. J. Appl. Physiol. 22, 461-468.

Feihl, F., Broccard, A.F., 2009. Interactions between respiration and systemic hemodynamics. Part I: basic concepts. Intensive. Care. Med. 35, 45-54.

Goldstein, D.S., Kopin, I.J., 2008. Adrenomedullary, adrenocortical, and sympathoneural responses to stressors: a meta-analysis. Endocr. Regul. 42, 111-119.

Holmes, C.L., Patel, B.M., Russell, J.A., Walley, K.R., 2001. Physiology of vasopressin relevant to management of septic shock. Chest. 120, 989-1002.

Jasnic, N., Korac, A., Velickovic, K., Golic, I., Djordjevic, J., Djurasevic, S., Djordjevic, I., Vujovic, P., Cvijic, G., 2010. The effect of acute heat exposure on rat pituitary corticotroph activation activation: the role of vasopressin. Folia. Histochem. Cytobiol. 48, 507-512.

Jochberger, S., Dörler, J., Luckner, G., Mayr, V.D., Wenzel, V., Ulmer, H., Morgenthaler, N.G., Hasibeder, W.R., Dünser, M.W., 2009. The vasopressin and copeptin response to infection, severe sepsis, and septic shock. Crit. Care. Med. 37, 476-482.

Katan, M., Fluri, F., Morgenthaler, N.G., Schuetz, P., Zweifel, C., Bingisser, R., Müller, K., Meckel, S., Gass, A., Kappos, L., Steck, A.J., Engelter, S.T., Müller, B., Christ-Crain, M., 2009. Copeptin: a novel, independent prognostic marker in patients with ischemic stroke. Ann. Neurol. 66, 799-808.

Katan, M., Christ-Crain, M., 2010. The stress hormone copeptin – a new prognostic biomarker in acute illness. Swiss. Med. Wkly. 140, w13101.

Koyner, J.L., Murray, P.T., 2010. Mechanical ventilation and the kidney. Blood. Purif. 29, 52-68.

Leclerc, F., Walter-Nicolet, E., Leteurtre, S., Noizet, O., Sadik, A., Cremer, R., Fourier, C., 2003. Admission plasma vasopressin levels in children with meningococcal septic shock. Intensive. Care. Med. 29, 1339-1344.

Leng, G., Brown, C.H., Russell, J.A., 1999. Physiological pathways regulating the activity of magnocellular neurosecretory cells. Prog. Neurobiol. 57, 625-655.

Lodha, R., Vivekanandhan, S., Sarthi, M., Kabra, S.K., 2006. Serial circulating vasopressin levels in children with septic shock. Pediatr. Crit. Care. Med. 7, 220-224.

Meijer, E., Bakker, S.J.L., van der Jagt, E.J., Navis, G., de Jong, P.E., Struck, J., Gansevoort, R.T., 2011. Copeptin, a surrogate marker of vasopressin, is associated with disease severity in autosomal dominant polycystic kidney disease. Clin. J. Am. Soc. Nephrol. 6, 361-368.

Mellbin, L.G., Ryden, L., Brismar, K., Morgenthaler, N.G., Öhrvik, J., Catrina, S.B., 2010. Copeptin, IGFBP-1, and cardiovascular prognosis in patients with type 2 diabetes and acute myocardial infarction. Diabetes. Care. 33, 1604-1606.

Morgenthaler, N.G., Struck, J., Alonso, C., Bergmann, A., 2006. Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. Clin. Chem. 52, 112-119.

Morgenthaler, N.G., Muller, B., Struck, J., Bergmann, A., Redl, H., Christ-Crain, M., 2007. Copeptin, a stable peptide of the arginine vasopressin precursor, is elevated in hemorrhagic and septic shock. Shock. 28, 219-226.

Müller, B., Morgenthaler, N., Stolz, D., Schuetz, P., Müller, C., Bingisser, R., Bergmann, A., Tamm, M., Christ-Crain, M., 2007. Circulating levels of copeptin, a novel biomarker, in lower respiratory tract infections. Eur. J. Clin. Invest. 37, 145-152.

Pannu, N., Mehta, R.L., 2007. Effect of mechanical ventilation on the kidney. Best. Pract. Res. Clin. Anaesthesiol. 18, 189-203.

Potocki, M., Breidthardt, T., Mueller, A., Reichlin, T., Socrates, T., Arenja, N., Reiter, M., Morgenthaler, N.G., Bergmann, A., Noveanu, M., Buser, P.T., Mueller, C., 2010. Copeptin and risk stratification in patients with acute dyspnea. Critical. Care. 14, R213.

Raff, H., Kane, C.W., Wood, C.E., 1991. Arginine vasopressin responses to hypoxia and hypercapnia in late-gestation fetal sheep. Am. J. Physiol. 260, R1077-R1081.

Rose, E.C. Jr., Godine, R.L. Jr., Rose, K.Y., Anderson, R.J., Carey, R.M., 1984. Role of arginine vasopressin and angiotensin II in cardiovascular responses to combined acute hypoxemia and hypercapnic acidosis in conscious dogs. J. Clin. Invest. 74, 321-331.

Schlapbach, L.J., Frey, S., Bigler, S., Manh-Nhi, C., Aebi, C., Nelle, M., Nuoffer, J.M., 2011. Copeptin concentration in cord blood in infants with early-onset sepsis, chorioamnionitis and perinatal asphyxia. BMC. Pediatr. 11, 38.

Schrier, R.W., Berl, T., Anderson, R.J., 1979. Osmotic and nonosmotic control of vasopressin release. Am. J. Physiol. 236, F321-F332.

Smith, D.W., Buller, K.M., Day, T.A., 1995. Role of ventrolateral medulla catecholamine cells in hypothalamic neuroendocrine cell responses to systemic hypoxia. J. Neurosci. 15, 7979-7988.

Stoiser, B., Mörtl, D., Hülsmann, M., Berger, R., Struck, J., Morgenthaler, N.G., Bergmann, A., Pacher, R., 2006. Copeptin, a fragment of the vasopressin precursor, as a novel predictor of outcome in heart failure. Eur. J. Clin. Invest. 36, 771-778.

Vasan, R.S., 2006. Biomarkers of cardiovascular disease: molecular basis and practical considerations. Circulation. 113, 2335-2362.

Wellmann, S., Benzing, J., Cippa, G., Admaty, D., Creutzfeldt, R., Arlettaz Mieth, R., Beinder, E., Lapaire, O., Morgenthaler, N.G., Haagen, U., Szinnai, G., Bührer, C., Bucher, H.U. 2010. High copeptin concentrations in umbilical cord blood after vaginal delivery and birth acidosis. J. Clin. Endocrinol. Metab. 95, 5091-5096.

Wood, C.E., Chen, H.G., 1989. Acidemia stimulates ACTH, vasopressin, and heart rate responses in fetal sheep. Am. J. Physiol. 257, R344-R349.

8. Figure legends

Figure 1

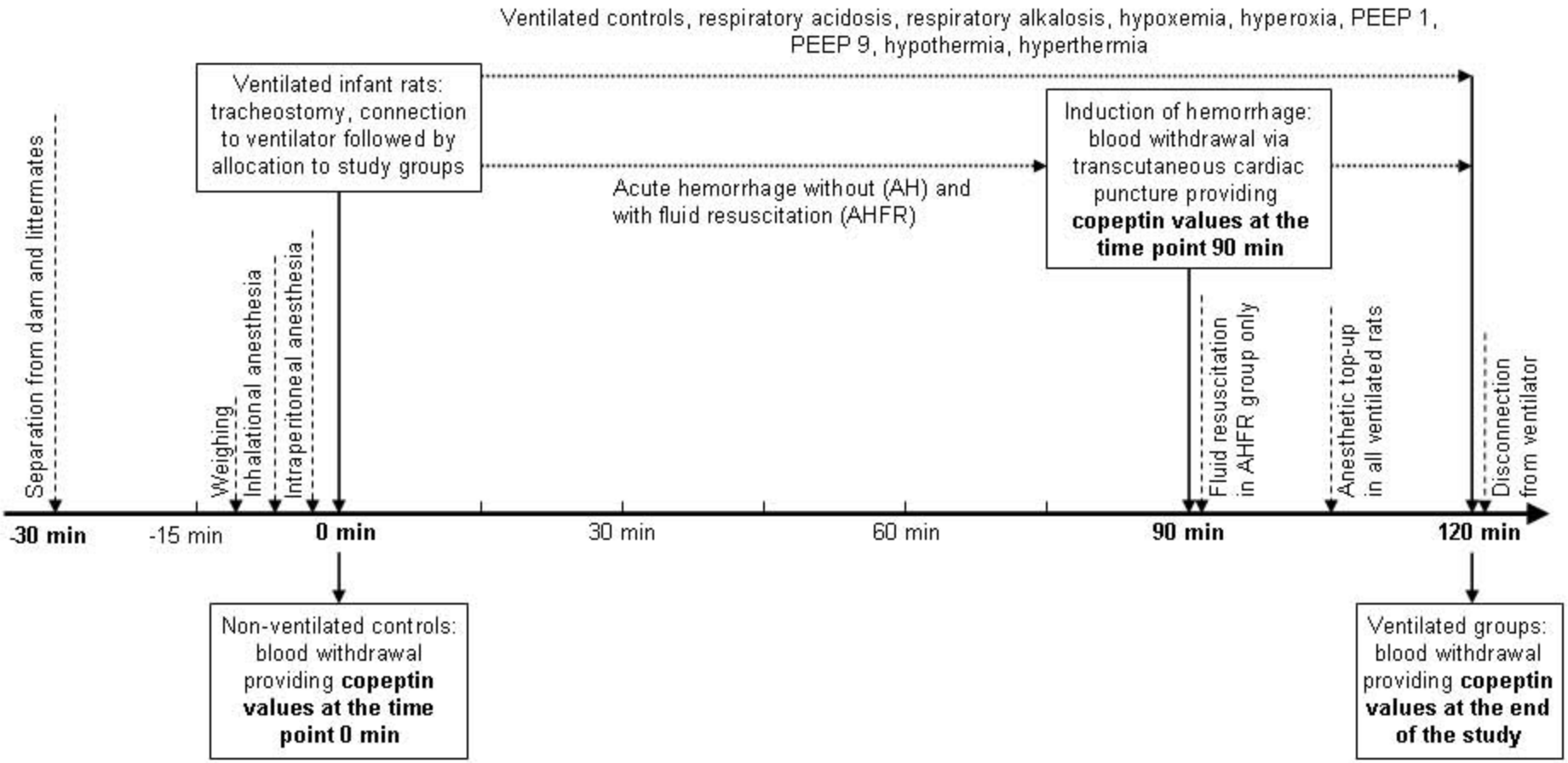
Experimental set-up illustrating the sequence of animal preparation and time points of interventions and blood withdrawal.

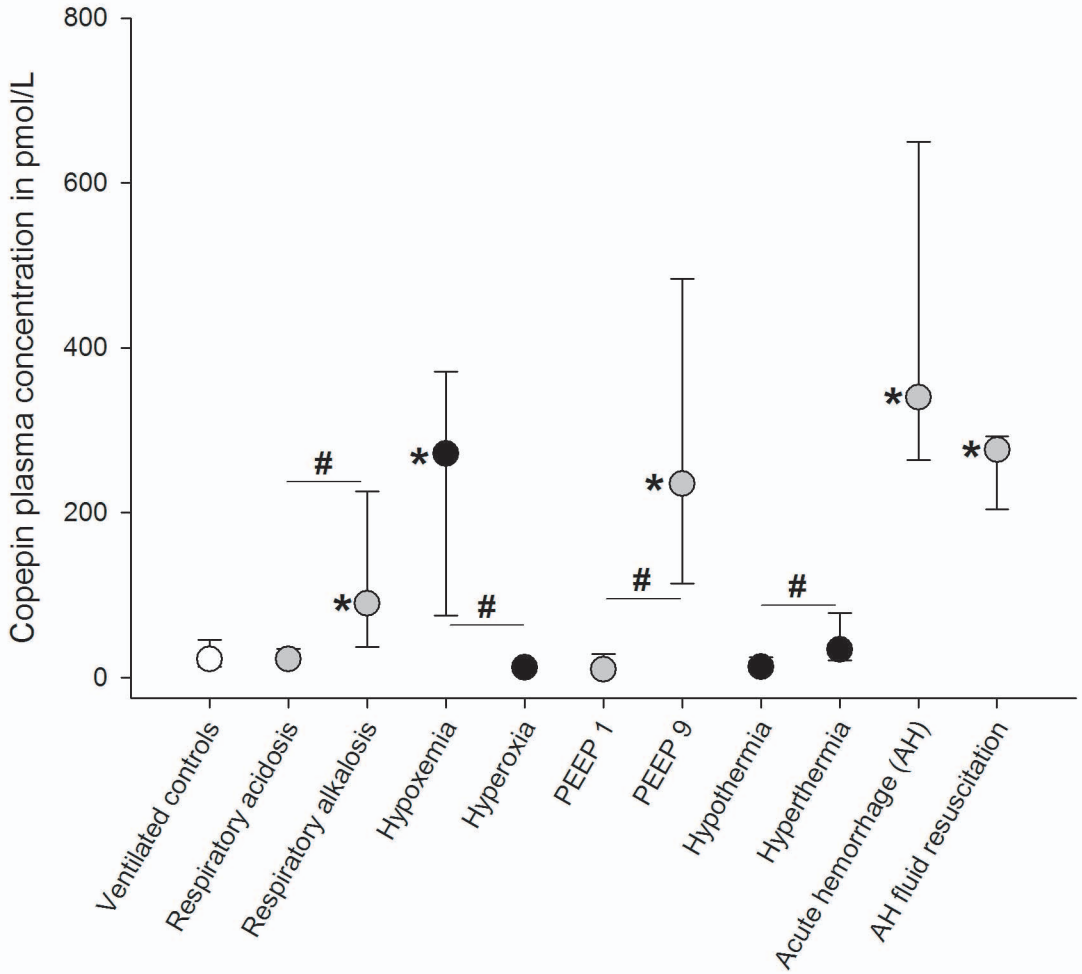
Figure 2

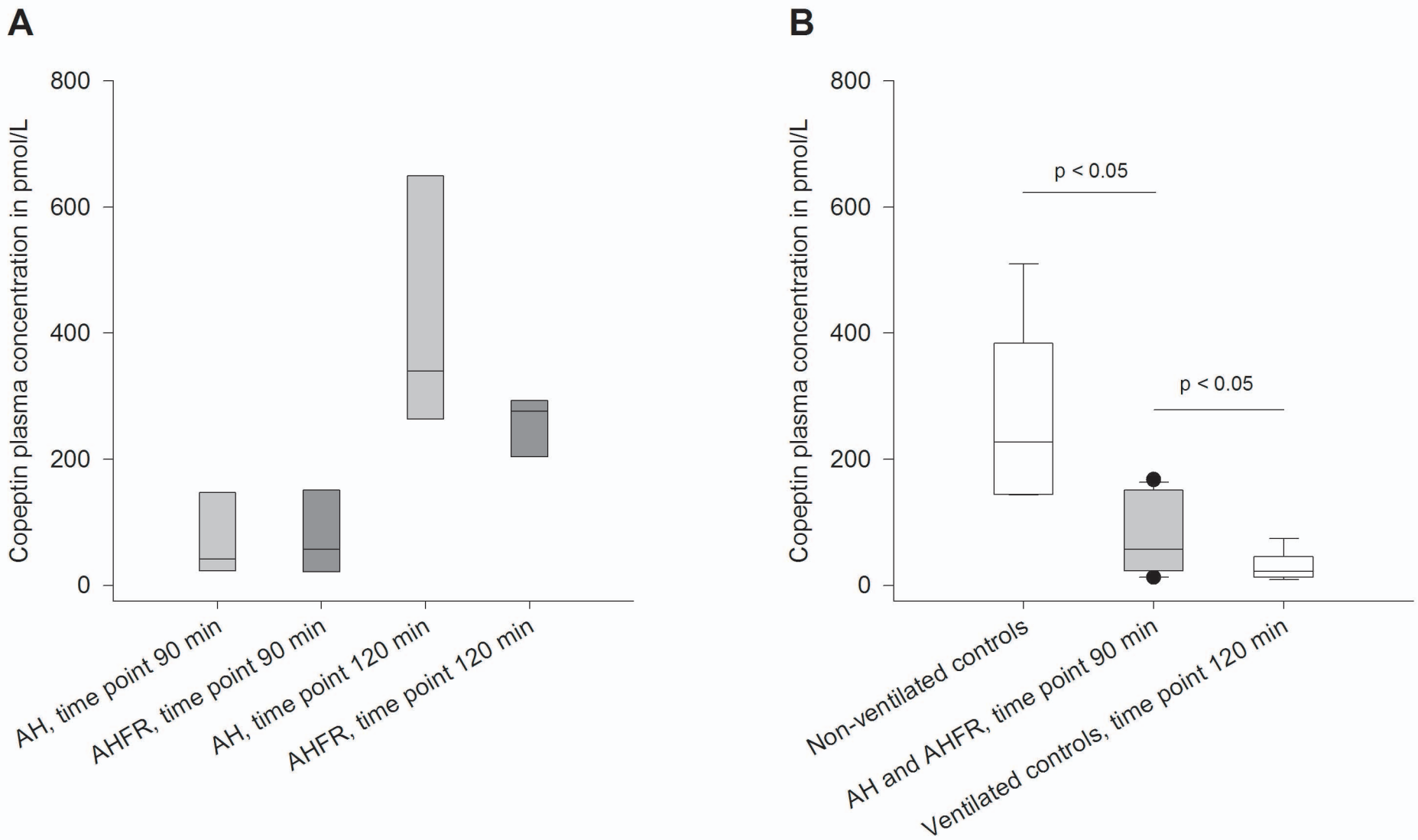
Copeptin plasma concentrations in ventilated study groups. All animals were mechanically ventilated for 120 min. Data are presented as simple error bars with median, 25^{th}, and 75^{th} percentiles. * indicates significant differences compared to ventilated control groups only; $^{\#}$ shows significant differences between pairs, e.g. respiratory acidosis versus alkalosis.

Figure 3

Panels A and B show copeptin plasma concentrations plotted against different time points. Data are displayed as vertical box plots with median, 10^{th}, 25^{th}, 75^{th}, and 90^{th} percentiles. Infant rats in group "AH" and "AHFR" were treated as "ventilated controls" up to the time point of 90 min, where acute hemorrhage (AH) was induced in both groups. Subsequently, only rats from group "AHFR" received fluid resuscitation. Both plasma obtained from induction of AH (time point 90 min) and plasma obtained 30 min later, i.e. at the time point 120 min, was used to measure copeptin. Panel A demonstrates a statistically significant increase of copeptin following AH. Fluid resuscitation had no impact on copeptin response ($p = 0.82$). Panel B displays copeptin half life.


Ventilated controls, respiratory acidosis, respiratory alkalosis, hypoxemia, hyperoxia, PEEP 1, PEEP 9, hypothermia, hyperthermia
Ventilated infant rats: tracheostomy, connection to ventilator followed by allocation to study groups
Acute hemorrhage without (AH) and with fluid resuscitation (AHFR)
Induction of hemorrhage: blood withdrawal via transcutaneous cardiac puncture providing **copeptin values at the time point 90 min**
Separation from dam and littermates
Weighing
Inhalational anesthesia
Intraperitoneal anesthesia
Fluid resuscitation in AHFR group only
Anesthetic top-up in all ventilated rats
Disconnection from ventilator
-30 min
-15 min
0 min
30 min
60 min
90 min
120 min
Non-ventilated controls: blood withdrawal providing **copeptin values at the time point 0 min**
Ventilated groups: blood withdrawal providing **copeptin values at the end of the study**


Copepin plasma concentration in pmol/L
800
600
400
200
0
*
#
Ventilated controls
Respiratory acidosis
Respiratory alkalosis
Hypoxemia
Hyperoxia
PEEP 1
PEEP 9
Hypothermia
Hyperthermia
Acute hemorrhage (AH)
AH fluid resuscitation


A
800
600
400
200
0
Copeptin plasma concentration in pmol/L
AH, time point 90 min
AHFR, time point 90 min
AH, time point 120 min
AHFR, time point 120 min
B
800
600
400
200
0
Copeptin plasma concentration in pmol/L
p < 0.05
p < 0.05
Non-ventilated controls
AH and AHFR, time point 90 min
Ventilated controls, time point 120 min

Table

Table 1

SpO_2, heart rate, peak and mean airway opening pressure (P_{ao}), pH, pCO_2, lactate, and urine output after 120 min of mechanical ventilation

Groups	SpO_2	Heart Rate	Peak P_{ao} (cmH_2O)	Mean P_{ao} (cmH_2O)	pH	pCO_2 (kPa)	Lactate (mmol/L)	Urine (mL)
Ventilated controls	98.5 (0.5)	292 (17)	12.3 (0.8)	8.4 (0.3)	7.42 (0.03)	5.2 (0.3)	0.8 (0.2)	0.3 (0.2)
Respiratory acidosis	98.2 (0.8)	281 (23)	#**11.4** (0.6)	*,#**7.8** (0.3)	*,#**7.25** (0.08)	*,#**8.0** (1.4)	#**0.7** (0.2)	0.3 (0.1)
Respiratory alkalosis	98.5 (0.5)	306 (18)	#**12.9** (1.1)	*,#**9.1** (0.5)	*,#**7.54** (0.07)	*,#**3.5** (0.7)	*,#**2.4** (1.1)	0.2 (0.1)
Hypoxemia	***77.6** (12.6)	297 (17)	12.0 (0.9)	8.3 (0.4)	7.39 (0.07)	5.2 (0.8)	*,#**2.0** (0.6)	0.3 (0.2)
Hyperoxia	98.9 (0.4)	291 (19)	12.8 (1.1)	8.7 (0.5)	7.38 (0.06)	6.1 (1.2)	#**0.8** (0.2)	0.3 (0.2)
PEEP 1	98.3 (0.2)	296 (13)	*,#**10.0** (1.2)	*,#**5.4** (0.5)	7.42 (0.03)	5.0 (0.3)	0.9 (0.1)	#**0.5** (0.2)
PEEP 9	98.6 (0.5)	284 (25)	*,#**18.6** (0.5)	*,#**13.4** (0.2)	7.41 (0.05)	4.9 (0.5)	1.1 (0.3)	#**0.1** (0.1)
Hypothermia	98.6 (0.6)	*,#**230** (43)	#**11.3** (0.8)	#**8.0** (0.3)	7.37 (0.07)	6.2 (1.2)	0.6 (0.1)	0.3 (0.2)
Hyperthermia	98.2 (0.8)	#**323** (20)	#**12.7** (0.8)	#**8.6** (0.4)	***7.30** (0.05)	5.9 (1.1)	#**1.5** (0.7)	0.2 (0.1)
Acute hemorrhage (AH)	98.6 (0.6)	281 (19)	12.8 (0.6)	8.7 (0.3)	7.33 (0.09)	5.2 (0.9)	***4.5** (1.6)	0.2 (0.1)
AH with fluids	97.9 (1.7)	275 (14)	13.2 (1.1)	8.8 (0.5)	7.32 (0.04)	5.0 (0.9)	***4.5** (2.0)	0.2 (0.1)

Data are expressed as group means ± standard deviation. * indicates statistically significant differences compared to ventilated controls only; # indicates statistically significant differences between pairs.